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Jørgensen, Louise von Gersdorff; Kania, Per; Sepúlveda, Dagoberto; Lorenzen, Niels; Stratmann, Ansgar; Buchmann, Kurt

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# A RECOMBINANT VACCINE TARGETING THE PARASITIC CILIATE *ICHTHYOPHTHIRIUS MULTIFILIIS*

**L. v. G. Jørgensen δ[1], P. W. Kania[1], D. A. S. Araneda[2], N. Lorenzen[2], A. Stratmann[3] and K. Buchmann[1]**

[1] Laboratory of Aquatic Pathobiology at the Department of Veterinary and Animal Sciences, University of Copenhagen, Frederiksberg, Denmark
[2] DTU AQUA, National Institute of Aquatic Resources, the Technical University of Denmark
[3] W42 Industrial Biotechnology GmbH, Dortmund, Germany

## Abstract

New vaccine candidates were identified targeting the one celled parasite *Ichthyophthirius multifiliis*, which negatively affects aquaculture freshwater fish productions all over the world. *In silico* selection with the use of artificial intelligence identified several potential vaccine candidates and three of these were recombinantly expressed using *E. coli* and insect cells. Following a vaccine trial one protein (a so-called neurohypophysial n-terminal domain protein, #10) was found to induce moderate protection against *I. multifiliis* in rainbow trout (*Oncorhynchus mykiss*). To develop a highly protective heterologous vaccine we aim to combine #10 with a protective epitope from the already known homologous protective antigen Iag52b, which is a GPI-anchored cysteine rich surface protein. To be able to produce #10 at low costs, recombinant expression has been conducted in an eukaryotic host. Purified Iag52b does not induce immunity in fish without the use of adjuvants, thus the most potentially protective epitope of Iag52 was selected *in silico* and coupled to a virus-like particle. This coupling enables the epitope to be presented in a virus-like conformation, which theoretically should be immunogenic to the fish.

## Experimental vaccination

The proteome of *Ichthyophthirius multifiliis* was screened using artificial intelligence *in silico* and 12 vaccine candidates were further investigated. Out of these, recombinant production was achieved for 7 proteins. Following a pilot study 3 proteins were selected as vaccine candidates.

**Subunit experimental vaccine**

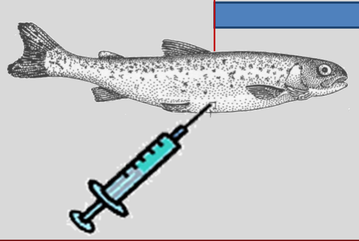
2 hypothetical proteins
1 neurohypophysial n-terminal domain protein

Alhydrogel
Freund's incomplete adjuvant

**Benchmark vaccine**

Disrupted parasites

Alhydrogel
Freund's incomplete adjuvant



Immunization — 4 wpi — 9,5 wpi — 13 wpi

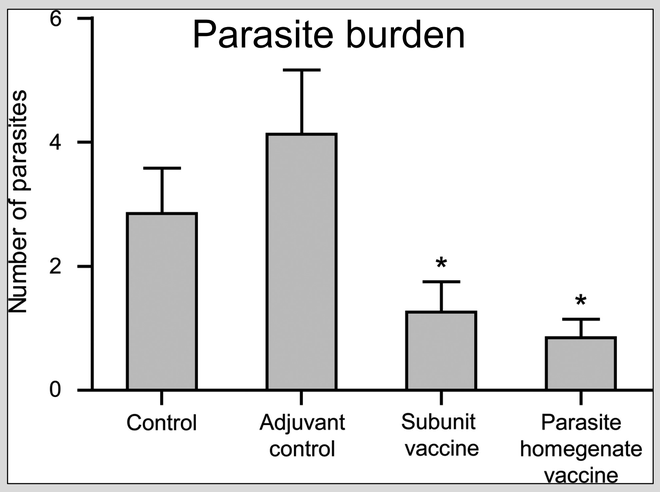
- Blood sampling
- ELISA

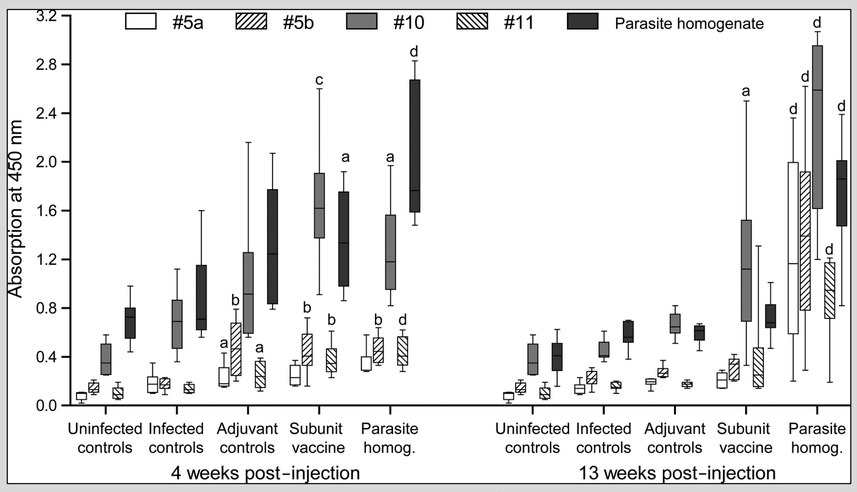
Challenge

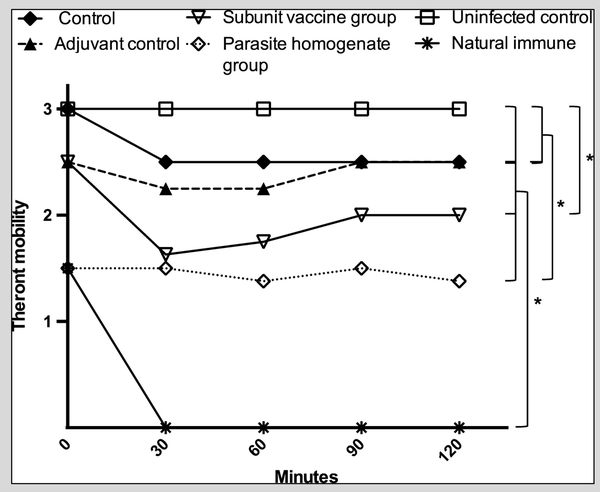
- Blood sampling
- Estimation of parasite burden
- Immobilization
- ELISA

A total of 190 fish were divided into five duplicated groups with 20 fish per replicate. Each fish in the recombinant vaccine group received 150 µL of vaccine consisting of equal amounts of alhydrogel and FIA with a mixture of the recombinant proteins bound to alhydrogel. Challenge was conducted by adding a suspension of theronts (10,000 theronts per fish) to the fish tank. Filtration was removed for the first 4 hours during challenge.

## Effect of the experimental vaccination

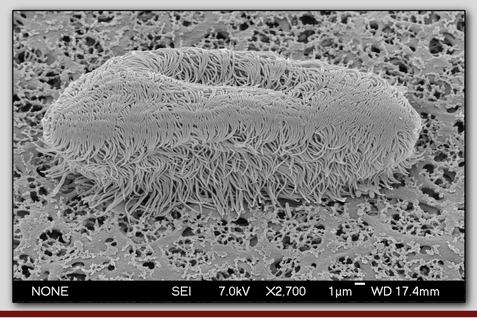



The **parasite burden** was significantly reduced in the subunit and the parasite homogenate vaccine groups. We estimated the protection to be moderate.

**ELISA** showed that the group of fish injected with the experimental vaccine and the parasite homogenate erected antibodies against one of the recombinant proteins, #10 (neurohypophysial n-terminal domain protein)




**Immobilization assay** conducted with live parasites incubated in plasma from vaccinated fish showed that the experimental vaccine and the parasite homogenate immobilized the parasites moderately.

## Improving immunogenicity of the vaccine

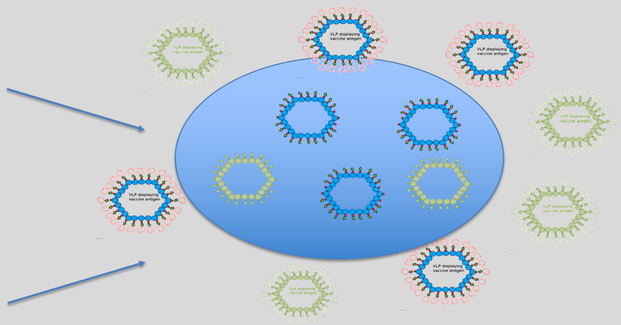
Plasmids

Sequence: **VLP** fused to Iag52b epitope

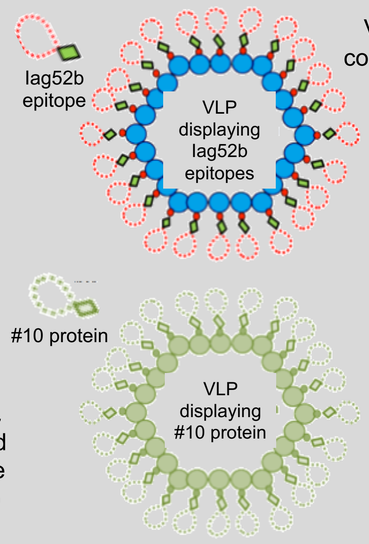
Sequence: VLP fused to #10 protein

**VLP** = Virus-Like Particle
VLPs are immunogenic and can possibly replace adjuvants

*Pichia pastoris* for recombinant expression



An already described protective antigen from the surface of *I. multifiliis*, called Iag52b, has not successfully been expressed recombinantly in conventional expression systems. Therefore we have identified two potentially protective epitopes *in silico* for recombinant expression and fusion with VLPs




The improved vaccine will contain VLPs displaying both Iag52b epitopes and #10 protein and will be injected with and without adjuvant. So far we have been able to produce VLPs with Iag52b epitopes to harvest in the supernatant from *Pichia pastoris* but protein #10 however is withheld within the cells. We are modifying signal sequences but If we are unable to target the protein to the supernatant, cell material will instead be used for the vaccine. The overall ambition is to develop a low cost recombinant vaccine that induce a high level of protection against the economically devastating parasitic ciliate *I. multifiliis* without the use of adjuvants in a wide range of fresh water fish species.

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